Proton toxicity interferes with the screening of common bean (Phaseolus vulgaris L.) genotypes for aluminium resistance in nutrient solution

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Summary—Zusammenfassung

Common bean (Phaseolus vulgaris L.) proved to be very sensitive of low pH (4.3), with large genotypic differences in proton sensitivity. Therefore, proton toxicity did not allow the screening of common bean genotypes for aluminium (Al) resistance using the established protocol for maize (0.5 mM CaCl₂, 8 μM H₂BO₃, pH 4.3). Increasing the pH to 4.5, the Ca²⁺ concentration to 5 mM, and addition of 0.5 mM KCl fully prevented proton toxicity in 28 tested genotypes and allowed to identify differences in Al resistance using the inhibition of root elongation by 20 μM Al supply for 36 h as parameter of Al injury. As in maize, Al treatment induced callose formation in root apices of common bean. Aluminium-induced callose formation well reflected the effect of Ca supply on Al sensitivity as revealed by root-growth inhibition. Aluminium-induced callose formation in root apices of 28 bean genotypes differing in Al resistance after 36 h Al treatment was positively correlated to Al-induced inhibition of root elongation and Al contents in the root apices. However, the relationship was less close than previously reported for maize. Also, after 12 h Al treatment, callose formation and Al contents in root apices did not reflect differences in Al resistance between two contrasting genotypes, indicating a different mode of the expression of Al toxicity and regulation of Al resistance in common bean than in maize.

Key words: aluminium / toxicity / resistance / genotypic differences / proton toxicity / callose formation / common bean

1 Introduction

Common bean (Phaseolus vulgaris L.) is the most important food legume for more than 300 million people, most of them in the developing world. It is the second source of protein in Eastern and Southern Africa and fourth in Tropical America, where it is also the third-most important calorlic source after cassava and maize (CIAT, 1999; Rao, 2001). Common bean is mainly produced on small-scale farms (80% of dry-bean production) in developing countries in Latin America and Africa, where about 40% of the bean-growing area is affected by Al toxicity, resulting in yield reductions from 30% to 60% (Thung and Rao, 1999; Wortman et al., 1998).

Acid soils comprise up to 40% of the world’s arable land (von Uexküll and Mutert, 1995), and soil acidity represents a major growth-limiting factor for plants (Foy, 1984). Although poor crop growth on acid soils is mainly correlated with Al³⁺ or H⁺ activities, other factors like manganese toxicity, low nitrogen supply, and deficiencies of phosphorus, calcium, magnesium, and molybdenum may also play a role (Foy, 1984; Rao et al., 1993).

Developing genotypes tolerant of acid soils is an ecologically friendly, energy-conserving, and economical solution for resource-poor farmers in the tropics. This genetic approach and the application of adapted agronomic practices in addition to maintenance lime applications are key factors for sustainable cropping systems on acid soils (Rao et al., 1993).

The relative impacts of Al and proton toxicities on plant performance in acid soils differ among plant species. The relative sensitivity to protons of three legume species increased in the order Pisum sativum < Glycine max < Phaseolus vulgaris (16%–65% inhibition of root elongation, respectively) when the pH of the nutrient solution was reduced from 6.0 to 4.05 (Lazof and Holland, 1999).

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Preliminary evaluation indicated significant genotypic variation in grain yield among bean genotypes grown on Al-toxic soils. These genotypic differences could be related to differences in Al resistance (Thung and Rao, 1999; CIAT, 1999). However, a systematic screening for Al resistance independently from soil factors other than Al toxicity requires a suitable screening procedure, which is not yet available for common bean. The development of such a technique would be facilitated by a better understanding of the physiological mechanisms responsible for Al resistance in common bean.

Solution-culture techniques allow to study the effects of one factor of the soil-acidity complex without affecting others, provided the adaptation of plant roots to low pH are considered (Edmeades et al., 1995). The present work aimed at developing the basic nutrient solution required to assess genotypic differences in Al resistance of common bean, independently of interactions with proton toxicity, and at evaluating the possible use of Al-induced callose formation to assess genotypic differences in Al resistance.

2 Materials and methods

2.1 Plant materials and growing conditions

The experiments were conducted with a set of 28 common bean (Phaseolus vulgaris L.) genotypes (Tab. 1), including land races and bred lines differing in adaptation to acid, Al-toxic soils. Seeds were provided by the International Center for Tropical Agriculture (CIAT), Cali, Colombia.

Common bean seeds were germinated for 3 d in plastic trays filled with peat limed to pH 5.5. Seedlings were carefully removed from the peat and the roots gently rinsed with distilled water to remove the attached peat. The seedlings were then transferred to a constantly aerated 22 L nutrient solution in pots equipped with an automatic pH titration device. The seedlings were grown for 24 h at pH 6.0 ± 0.2, followed by lowering the pH in steps of 0.3 pH units until the target pH was reached after 18 h. Thereafter, the pH was kept constant by adding 0.1 M HCl or 0.1 M KOH. Plants were grown under controlled environmental conditions in a growth chamber with a 16 h/8 h light/dark regime, 27°C/25°C day/night temperatures, 70% relative air humidity, and a photon flux density of 230 μmol m⁻² s⁻¹ photosynthetic active radiation at plant height.

To verify the suitability of the nutrient solution medium used for maize (Horst et al., 1997; Collet et al. 2002; Wang et al., 2004; Eticha et al., 2005) for common bean, seedlings of the genotypes SEA-5 and VAX-1 were grown at 0.5 mM CaCl₂ and 8 μM H₃BO₃ at pH 6.0, 4.5, and 4.3. Six hours after the target pH was reached, the plants were treated with nominal Al concentrations of 0, 10, and 15 μM as AlCl₃ for up to 36 h. Seedlings of the genotypes SEA-5 and VAX-1 were further grown at pH 6.0 and 4.5 in nutrient solutions containing 8 μM H₃BO₃ and different KCl (0 and 0.5 mM) and CaCl₂ concen-

Table 1: List of the common bean genotypes used in the Al-screening experiments.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Classification</th>
<th>Notes</th>
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<tbody>
<tr>
<td>A-774</td>
<td>Breed</td>
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2.2 Determination of the growth of roots and their callose and Al contents

Root elongation was determined by measuring the primary root length at the beginning and 36 h after Al treatment using a 1 mm scale keeping the root submerged in nutrient solution. The difference between the initial and the final length during the treatment period was defined as root-elongation rate (RER). Aluminium-induced inhibition of root elongation was calculated as:

\[
\text{Al-inhibited root elongation} \left[\%\right] = \frac{\text{RER}_{\text{control}} - \text{RER}_{\text{Al}}}{\text{RER}_{\text{control}}} \times 100
\]

RER\text{control} - root-elongation rate at 0 \text{ µM Al}

RER\text{Al} - root-elongation rate at 20 \text{ µM Al}

After treatment, roots were rinsed with distilled water, and 10 mm root tips were excised using a razor blade, stored in Eppendorf tubes (Eppendorf AG, Hamburg, Germany), and fixed immediately in liquid nitrogen. The root tips were homogenized in 500 µL of 1 M NaOH with a mixer mill (MM 200, Retsch GmbH & Co. KG, Haan, Germany) at a speed of 20 cycles s⁻¹ for 2 min. After homogenization, another 500 µL of 1 M NaOH were added, and callose was solubilized by heating in a water bath at 80°C for 20 min. Thereafter, samples were centrifuged at 21000 g for 5 min. Callose was measured according to Kauss (1989) after addition of aniline blue reagent, using a Microplate Fluorescence Reader (Fix 800, Bio-Tek Instruments Inc, Winooski, Vermont, USA), at excitation and emission wavelengths of \(\lambda = 400/30\) and \(\lambda = 485/40\) nm, respectively, sensitivity 50. Pachyman (1,3-β-D-glucan, Calbiochem, Deisenhofen, Germany) was used as calibration standard. Hence, callose contents were expressed as pachyman equivalents (PE) per root tip.

After treatment, roots were rinsed with distilled water and 10 mm root tips were excised using a razor blade, stored in Eppendorf cups and kept at 4°C. Samples were placed in teflon centrifuge tubes and digested in 500 µL ultrapure HNO₃ (65% v/v) in a Microwave-Laboratory-System (MLS-ETHOS Plus, MLS GmbH, Leutkirch, Germany) for 3 h. Thereafter, the volumes of the samples were adjusted to 2 mL with ultrapure water. Aluminium in the samples was analyzed by ICP-OES (Spectro Analytical Instruments GmbH, Kleve, Germany) at a wavelength of \(\lambda = 308.21\) nm.

2.3 Experimental design and statistical analysis

Completely randomized designs were used in all experiments with four or eight replicates, depending on the experiment. After analysis of variance (Proc GLM), the means were compared using the Tukey test. Data were statistically analyzed using SAS 8 (SAS Institute Inc., Cary, NC, USA). *, **, *** denote significant differences at \(p < 0.05, 0.01, 0.001\), respectively; ns, nonsignificant.

3 Results

3.1 Adaptation of the basal incubation medium used for maize to common beans

When the solution pH decreased from pH 6.0 to 4.3, the root-elongation rate was significantly inhibited in both genotypes tested (Fig. 1a). In general, growth inhibition was more...
severe in VAX-1 than in SEA-5. At pH 4.5, root growth of SEA-5 was less affected (12% reduction compared to pH 6.0) than of VAX-1 (58% reduction compared to pH 6.0). At pH 4.3, root-elongation rates of both genotypes were greatly reduced (74% and 85% for SEA-5 and VAX-1 compared to pH 6.0, respectively). Al supply additionally inhibited root-elongation rate (Fig. 1b). However, significant differences were only observed at pH 4.5. Treatment with 10 μM Al reduced root growth of genotypes SEA-5 and VAX-1 by more than 74% and 80%, respectively. At pH 4.3, the control roots particularly of VAX-1 were too much damaged by H⁺ toxicity to show a clear Al effect.

3.2 Modifications of the basal incubation medium

Addition of K (0.5 mM) to the basal nutrient solution (0.5 mM CaCl₂ and 8 μM H₃BO₃) fully prevented the observed reduction in the root growth of SEA-5 grown at pH 4.5 (Fig. 2). Likewise, K supply enhanced the root growth of VAX-1 (cf., Fig. 1a). However, complete recovering of the root growth (relative to pH 6.0) was only achieved at 5 mM CaCl₂ (Fig. 3a). Genotype SEA-5 showed higher root-elongation rates than VAX-1, independent of pH and Ca concentration.

Aluminum application (10 μM Al) strongly inhibited root elongation of both genotypes similarly at low Ca supply (Fig. 3b) and K supply (Fig. 2). Increasing the Ca supply improved root growth of the controls (Al 0) only in VAX-1. However, in the presence of Al, increasing the Ca supply greatly improved root growth, suggesting a reduction of Al toxicity by Ca in both genotypes. At 1 mM and particularly at 5 mM Ca supply, clear genotypic differences in Al resistance appeared: SEA-5 proved to be more Al-resistant than VAX-1. This differentiation was best at 20 μM Al supply.

The callose and Al contents of the controls (Al 0) were low and independent of the pH of the nutrient solution (not shown). Thus, proton toxicity did not induce callose formation in bean. At 0.5 mM Ca supply, even the lowest Al supply of 10 μM strongly increased callose contents in the root apices (Fig. 4a). A higher Al supply only slightly further enhanced callose formation. Increasing the Ca supply particularly

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increased the callose formation induced by the lower Al supplies. However, at 50 µM Al supply, callose contents were high, independent of the Ca supply. Al-induced callose contents well reflected Al-induced inhibition of root elongation (Fig. 5a) in this experiment, where the Ca supply was the main factor determining the Al response of both genotypes. However, the greater Al resistance of SEA-5 (as indicated by less Al-induced root elongation) was not reflected in lower callose contents. Particularly at 5 mM Ca supply, which best differentiated the genotypes regarding their level of Al resistance, Al-resistant SEA-5 produced significantly more callose than Al-sensitive VAX-1.

The Al content of the root apices increased with Al supply and decreased with Ca supply (Fig. 4b), reflecting competition between Al³⁺ and Ca²⁺ for root uptake/binding. Significant differences between genotypes and Al treatments were observed at all Ca levels. In general, the Al content reflected Al-induced inhibition of root elongation ($R^2 = 0.45$; Fig. 5b). However, the greater Al resistance of SEA-5 (see above) was not reflected in a lower Al content, especially at 5 mM Ca supply. The Al contents in the root tips were positively correlated with callose formation ($R^2 = 0.74$).

### 3.3 Confirming the lack of proton (H⁺) toxicity at pH 4.5

As shown above, the two genotypes differed substantially in their sensitivity of low pH. The modification of the nutrient solution by increasing the pH to 4.5 and the Ca supply to 5 mM and the addition of 0.5 mM K ameliorated the proton toxicity in the Al-sensitive genotype VAX-1. To confirm that this treatment generally prevents proton toxicity in all bean genotypes to be screened for Al resistance, we compared the root growth of 28 genotypes at pH 6 and pH 4.5 using the modified nutrient solution (Fig. 6). The genotypes differed significantly in root-elongation rate, independent of the solution.

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**Figure 4:** Effect of different Al and Ca supplies on Al-induced callose formation (a) and Al contents (b) in 10-mm root tips of two bean genotypes grown for 12 h in a solution containing 0.5 mM KCl and 8 µM H$_3$BO$_3$, pH 4.5. Bars represent means ± SD (n = 4).

**Figure 5:** Relationships between Al-inhibited root elongation and Al-induced callose formation (a), Al-inhibited root elongation and Al content (b), and Al-induced callose formation and Al content (c) of two common bean genotypes grown in a solution containing 0.5 mM KCl and 8 µM H$_3$BO$_3$ at different Ca and Al supplies, pH 4.5. Callose and Al contents in 10-mm root tips were measured in plants treated for 12 h. Al-inhibited root elongation was measured in plants treated for 36 h (n = 4); *** denotes significance at $p < 0.001$.

**Abbildung 4:** Einfluss des Al- und Ca-Angebotes auf die durch Al induzierten Kallose- (a) und Al-Gehalte (b) in 10-mm-Wurzelspitzen von zwei Buschbohnen-Genotypen nach 12 h Kultur in einer Nährösung, die 0.5 mM KCl und 8 µM H$_3$BO$_3$ enthielt, pH 4.5. Die Balken zeigen Mittelwerte ± SD (n = 4).

**Abbildung 5:** Beziehungen zwischen Al-induzierter Hemmung des Wurzelwachstums und Al-induzierter Kallose-Bildung (a), Al-induzierter Hemmung des Wurzelwachstums und Al-Gehalten (b) sowie Al-induzierter Kallosebildung und Al-Gehalten (c) bei zwei Buschbohnen-Genotypen nach Kultur in einer Nährösung, die 0.5 mM KCl und 8 µM H$_3$BO$_3$ enthält, pH 4.5, bei variertem Al- und Ca-Angebot. Die Kallose- und Al-Gehalte in 10-mm-Wurzelspitzen wurden nach 12 h, Al-induziertes Wurzelwachstum nach 36 h Behandlungsduer bestimmt (n = 4); *** zeigt Signifikanz bei $p < 0.001$ an.
Based on the previous results, 28 common bean genotypes were screened for Al resistance in a solution with 5 mM Ca, 0.5 mM K, 8 mM KCl, and 8 mM H$_3$BO$_3$. The effect of Al on root-elongation rate is best shown as Al-induced inhibition of root elongation (Fig. 7). The genotypes showed high significant differences in response to Al supply. The genotypes were arbitrarily ranked for Al resistance in three categories, based on the percentage of Al-induced inhibition of root elongation. Accordingly, nine genotypes were classified as Al-sensitive (inhibition >50%) with VAX-1, MAR-1, and DOR-714 being the most Al-sensitive genotypes. Ten genotypes were classified as intermediate (inhibition between 50% and 30%), and seven genotypes were classified as Al-resistant (inhibition <30%). Among these latter seven, three Andean genotypes (G-5273, Quimbaya, and BRB-19B) showed outstanding levels of Al resistance.

4 Discussion

4.1 Adaptation of the basal incubation medium used for maize to common bean

In the present study, the basal incubation medium commonly used for maize was tested for common bean genotypes. The reduction of the pH from 6.0 to 4.3, which is the standard pH used for the Al-screening in maize (Horst et al., 1997), greatly inhibited the root elongation of bean genotypes in the absence of Al. This makes it difficult to select for Al resistance, because of the lack of a proper control (Fig. 1a).
is in agreement with Lazof and Holland (1999), who clearly showed the high proton sensitivity of two common bean genotypes. They concluded that screening bean for Al resistance is not possible at pH 4.05 and proposed to evaluate recovery from Al stress as indicator of Al resistance. Because of the high proton sensitivity of common bean, Massot et al. (1990) classified six genotypes for Al resistance using a solution pH of 4.8, but without control of pH and also without monitoring Al activity in solution. The high proton sensitivity of common bean may be related to a low efficiency of the plasma-membrane proton ATPase (Yan et al., 1992, 1998), and thus the inability to maintain the cytosolic pH leading to cell injury (Schubert and Yan, 1997).

Calcium and other polyvalent cations play a crucial role in maintaining the integrity of the ion-absorption process, especially in the acid pH range (Moore, 1971). Calcium concentrations in the nutrient solution (0.5 mM) have been proven to be sufficient to avoid proton toxicity in maize, but not in common bean (Fig. 1a). From their experiments conducted with maize and broad bean, Yan et al. (1992) concluded that the Ca\(^{2+}\) requirement to recover root growth at a given low pH was smaller for maize than for broad bean. In our experiments with common bean, a similar root growth at pH 4.5 as at pH 6.0 was observed with addition of 5 mM Ca\(^{2+}\) (Fig. 3a). Since we did not use Ca concentrations between 1 and 5 mM, we cannot exclude that Ca concentrations of less than 5 mM

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**Figure 8:** Al-induced callose formation (a) and Al contents (b) in 10 mm root tips of 28 common bean genotypes grown in a solution containing 5 mM CaCl\(_2\), 0.5 mM KCl, and 8 \(\mu\)M H\(_2\)BO\(_3\) for 36 h at 20 \(\mu\)M Al, pH 4.5. Genotypes are arranged from left to right according to increasing Al resistance based on Al-inhibited root elongation (Fig. 7). Bars represent standard deviations from the means (n = 4).

**Abbott 8:** Al-induzierte Kallose- (a) und Al-Gehalte (b) in 10-mm-Wurzelspitzen von 28 Buschbohnen-Genotypen nach Kultur für 36 h in einer Nährösung, die 5 mM CaCl\(_2\), 0.5 mM KCl, 8 \(\mu\)M H\(_2\)BO\(_3\) und 20 \(\mu\)M Al enthielt, pH 4,5. Die Genotypen sind von links nach rechts angeordnet, entsprechend ihrer auf der Al-induzierten Hemmung des Wurzelwachstums basierenden Al-Resistenz (Abb. 7). Die Balken zeigen Mittelwerte ± SD (n = 4).

**Figure 9:** Relationships between Al-inhibited root elongation and Al-induced callose formation (a), Al-inhibited root elongation and Al content (b), and Al-induced callose formation and Al content (c) of 28 common bean genotypes grown in a solution with 5 mM CaCl\(_2\), 0.5 mM KCl, and 8 \(\mu\)M H\(_2\)BO\(_3\) for 36 h at 20 \(\mu\)M Al, pH 4.5 (n = 4). *** denotes significance at p < 0.001.

**Abbott 9:** Beziehungen zwischen Al-induzierter Hemmung des Wurzelwachstums und Al-induzierter Kallose-Bildung (a), Al-induzierter Hemmung des Wurzelwachstums und Al-Gehalten (b) sowie Al-induzierter Kallose-Bildung und Al-Gehalten (c) bei 28 Buschbohnen-Genotypen nach Kultur für 36 h in einer Nährlosung, die 5 mM CaCl\(_2\), 0.5 mM KCl, 8 \(\mu\)M H\(_2\)BO\(_3\) und 20 \(\mu\)M Al enthielt, pH 4,5 (n = 4); *** zeigt Signifikanz bei p < 0.001 an.
may have been sufficient. The alleviation of H⁺ toxicity by Ca²⁺ can be attributed to the displacement of H⁺ from the cell wall and outer face of the plasma membrane, thus maintaining cell-wall (Koyama et al., 2001) and plasma-membrane (Hanson, 1984) properties, and the H⁺ release from the cytosol through the H⁺-ATPase, which is a prerequisite for root growth (Kiraide, 1998; Yan et al., 1992). Potassium (Fig. 2) has been shown to increase the activity of the H⁺-ATPase (K⁺ antiport) by increasing the affinity of the ATPase for ATP, therefore enhancing root growth (Lindberg and Yahya, 1994). The modified nutrient solution (5 mM Ca²⁺ and 0.5 mM K⁺) resulted in equal root-elongation rates of bean genotypes at pH 6.0 and 4.5 (Fig. 6), thus obtaining a proper control for the Al treatments.

Al treatment caused a significant increase of callose formation in root tips of bean genotypes (Fig. 4a). Within the range of 6.0 to 4.3, no effect of pH on callose formation was observed. The best differentiation in callose formation between genotypes was obtained at the highest Ca²⁺ supply. Callose served. The best differentiation in callose formation between pH 6.0 and 4.5 (Fig. 6), thus obtaining a proper control for the Al treatments.

Al treatments caused a significant increase of callose formation in root tips of bean genotypes (Fig. 4a). Within the range of 6.0 to 4.3, no effect of pH on callose formation was observed. The best differentiation in callose formation between genotypes was obtained at the highest Ca²⁺ supply. Callose formation is one of the earliest physiological reactions of roots to Al stress and a sensitive parameter of Al injury in soybean (Wissemieier et al., 1992; Staß and Horst, 1995), wheat (Zhang et al., 1994), maize (Collet, 2001), and bean (Massot et al., 1999). According to current knowledge, callose formation is initiated through changes in plasma-membrane fluidity and permeability (Jones and Kochian, 1995) and increased cytosolic Ca²⁺ activities (Rengel and Zhang, 2003; Sivaguru et al., 2005). Staß and Horst (1995) also observed no effect of pH on callose induction when soybean cells were grown in the pH range between 4.3 and 7.0. Wissemieier and Horst (1995) found a reduction in the callose formation in soybean plants with increasing Ca²⁺ levels in the nutrient solution. They also demonstrated the necessity of sufficient Ca²⁺ in the external solution to promote Al-induced callose formation.

Calcium amelioration of Al toxicity as reflected by lower root-growth inhibition (Fig. 3b) and lower callose formation (Fig. 4a) can be related to decreased Al concentrations in the root apices (Fig. 4b, Fig. 5). However, even at 50 μM Al supply, Al toxicity could not be prevented, confirming that Al toxicity cannot be explained by Al-induced Ca deficiency (Ryan et al., 1994).

4.2 Screening for Al resistance of common bean genotypes based on root elongation and callose formation

Using the modified Al solution allowed for the identification of significant differences in the Al resistance among the 28 genotypes on the basis of Al-inhibited root elongation (Fig. 7). This shows the existence of a genetic variability in response of common bean to Al stress and underlines the possibility of using the methodology for the screening of a larger set of genotypes. Aluminium-resistant genotypes like G-5273 (Andean) and G-21212 (Mesoamerican) have been found to be outstanding in their performance under acid soil conditions. However, genotypes VAX-1 and MAR-1, which were classified as highly Al-sensitive based on relative root elongation in nutrient solution, performed very well on acid soils (CIAV, 1999, 2000). The superior performance of VAX-1 under acid soil conditions was associated with abundant basal and adventitious-root development (Rao et al., 2004).

However, Al toxicity may not have been the only growth-limiting factor on the acid soils used for the field tests. P-acquisition efficiency (Baligar et al., 1997; Shen et al., 2002a), adaptation to low levels of Ca and Mg, and establishment and maintenance of the N₂-fixing rhizobia symbiosis (Rao, 2001) may well have been equally or even more important. Also, Horst and Kloet (1990) reported a poor correlation between Al resistance of 31 soybean genotypes in solution and sand cultures. This indicates that in substrate genotypic Al resistance may be modified by root exudation as suggested by Horst et al. (1990).

Callose synthesis has been demonstrated to be a sensitive short- (1–8 h) and short- and medium-term (8–24 h) marker for Al injury in soybean (Wissemieier et al., 1992; Staß and Horst, 1995) and maize (Horst et al., 1997; Kollmeier et al., 2000; Collet et al., 2002), respectively. A decrease in Al-induced callose contents in root apices after 22 h Al treatment has been reported for soybean (Wissemieier and Horst, 1995) and after 24 h for maize (Collet, 2001). A decrease in the callose content through depolymerization of callose by (1,3)-β-glucanase has been suggested by Wissemieier and Horst (1995) to be the mechanism explaining the low callose contents in bean root apices found after 36 h Al treatment (Fig. 8a).

Differences in Al contents and thus callose contents of root tips between genotypes after medium- and longer-term (36 h) Al supply may be the result rather than the cause of differences in root-growth rate: Al-resistant genotypes maintained root growth and thus “diluted” Al and callose contents. Also, lower Al contents in Al-resistant genotypes may result from Al exclusion through the release of organic-acid anions (Shen et al., 2002b) after an Al-induction period typical for Patter II response plants (Ma et al., 2001). This may explain why after 36 h of Al treatment, significant correlations were found between callose and Al contents and Al-induced inhibition of root elongation (Fig. 9). However, much closer correlations were found with Ca supply and solution pH as the main sources of variation after a medium-term (12 h) exposure to Al (Fig. 5), which drastically varied the severity of the Al stress. Nevertheless, genotypic differences in Al resistance were not reflected in corresponding differences in Al-induced callose formation and Al contents in root apices (Fig. 4). This is in contrast to results obtained with maize, where short-term Al supply induced callose contents in root apices as a most suitable indicator of Al sensitivity, which may be used for the characterization of inheritance of Al resistance and as a screening tool for adaptation to acid, Al-toxic soils (Eticha et al., 2005). It thus appears that the expression of Al toxicity and the resistance in common bean differ from that of maize. Therefore, more detailed studies on the kinetics of Al-induced inhibition of root elongation, Al-induced callose formation in relation to the release of organic acid anions, and the accumulation of organic acids and Al are necessary to better understand genotypic differences in Al resistance and to develop quick screening techniques for Al resistance.
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**References**


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